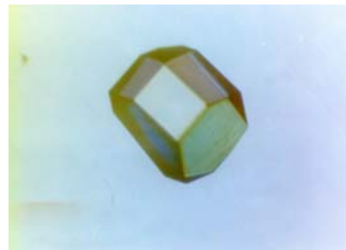
biophysical tools to improve crystallisation

Helena Berglund

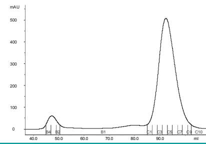
biophysics

- biophysics?
- characterisation and screening to optimize protein stability and homogeneity
- methods at SGC Stockholm



characterisation starts during purification

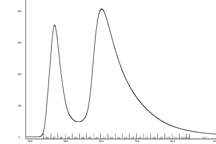
1



nice symmetric peak

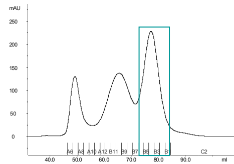
continue as usual

2



single non-symmetric peak
continue as usual

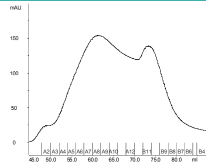
3



desired protein in several separated peaks

continue with the lowest mol weight peak (unless other knowledge about multimeric state)

4,5

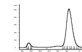


4: additional humps originate from contaminating proteins

5: just broad and ugly

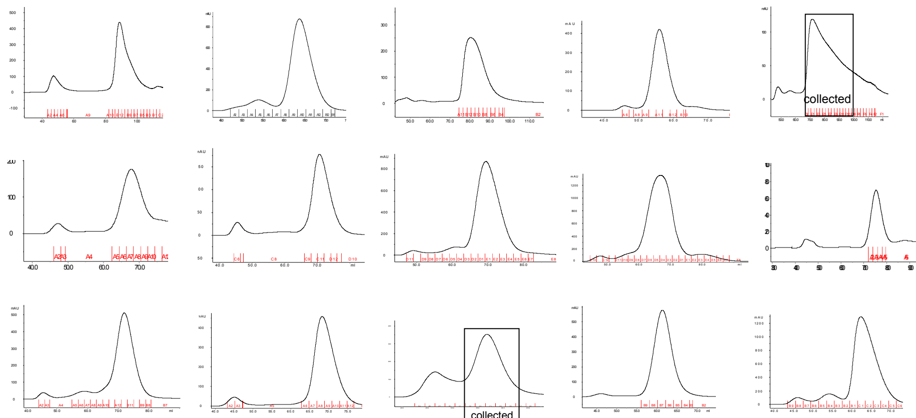
gel filtration elution profiles

All structure giving proteins so far from GF profile :

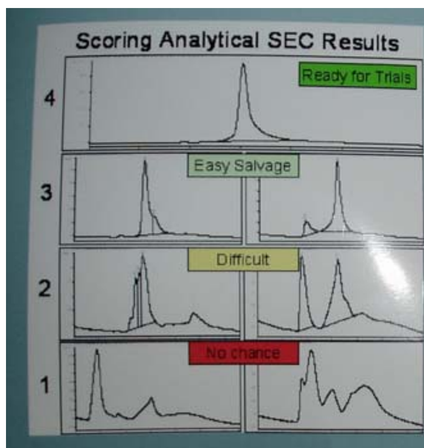
majority: 1 

occationaly: 2 3

gel filtration elution profiles for SGC Stockholm's first structure determined proteins:
single symmetric peaks, eluted at expected retention volume.



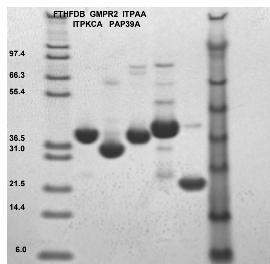
poster from JCSG at ICSG Beijing 2006



SEC score	solved structures (total 118)
4	83%
3	6%
2	0
1	0
?	11%

characterisation of all batches

- SDS-PAGE- purity and chemical homogeneity
  - contaminating proteins
  - size heterogeneities
  - follow up: re-purification



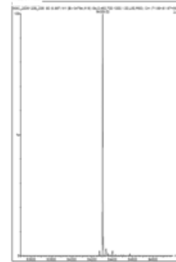
characterisation of all batches

- Mass spectrometry- chemical homogeneity
  - verify the identity
  - chemical homogeneity
  - investigate Sel-Met incorporation etc

  - performed at KTH
    (Gustav Sundqvist and Harry Brumer, School of Biotechnology)

  - March -07:
    1220 samples
    940 correct
    190 not correct
    90 no result

  - follow up: e.g. sequencing



mass spectrometry

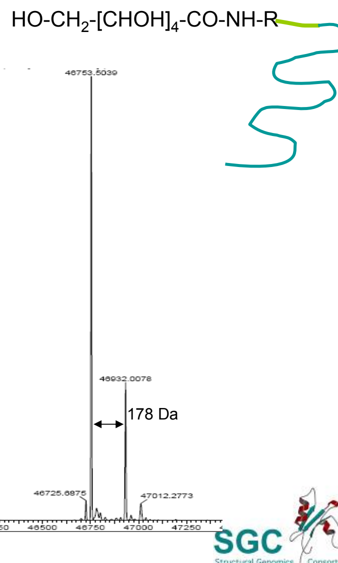
detected features:

- point mutations
  - usually in primer regions
- construct/sample mix-ups
- bound detergent molecules
- arg-lys substitutions
  - differential usage of codons in humans and *E. coli*
  - *E. coli* might incorporate a lysine (AAA) if there is a lack of correct arg-tRNA.
  - follow up : express the protein in *E.coli* strain with extra supply of tRNAs for the rare codons

mass spectrometry

another detected feature:

- His tag modification
    - α-N-gluconoylation
    - degree of modification dependent on the His tag sequence used:
    - frequent in MGSSHHHHHH...
    - not so frequent in MHHHHHH.....



Geoghegan et al, Analytical Biochemistry 267 169-184 1999

SGC Structural Genomics Consortium

mass spectrometry

co-purifying proteins from the IMAC step:

we see SlyD and GroEL regularly

Table 1
Native proteins from *E. coli* commonly co-purified during IMAC

Protein	SwissProt access code	Molecular Mass (kDa)	% Histidine residues	Isoelectric point (pI)	Metal requirement
Fur	P06975	16.7	8.1	5.6	Fe^{2+}, Zn^{2+} [a]
YodA	P76344	22.3	5.2	5.6	Cd^{2+}, Zn^{2+} [a]
Cu-Zn-SODM	AAC74718	17.6	4.0	5.9	Cu^{2+} [a], Zn^{2+} [a]
ArgE	P23908	42.3	4.4	5.5	Fe^{2+}, Ni^{2+}
YadF	P36857	25.0	5.5	6.1	Zn^{2+}, Hg^{2+} [a,b]
GlgA	P08323	51.7	3.4	6.0	Mg^{2+} [a,c]
GlmS	P17169	66.8	3.9	5.5	
CAT	AAA57080	25.5	5.5	5.9	Co^{2+}
Crp	P03020	23.6	2.9	8.3	
Hfq	P25521	11.1	4.9	6.9	
→ SlyD	P30856 20850 Da	20.8	10.2	4.8	Zn^{2+}, Ni^{2+}
S15	P02371	10.2	5.6	10.4	
YfbG	P77398	74.2	4.1	6.3	
→ Hsp60	AAC77103 57195 Da	57.0	0.2	4.8	
ODO1	P07015	10.5	3.6	6.0	
ODO2	P07016	44.0	1.7	5.5	
G6PD	P22992	55.7	1.2	5.5	

The table summarises the physicochemical properties of these proteins.
[a] Metal ions reported to be present in the crystallization solution.
[b] As seen in the structure of its human counterpart (PDB: 1CRM).
[c] Observed in its human counterpart (PDB 1PYX).

V.M. Bolanos-Garcia, O.R. Davies / Biochimica et Biophysica Acta 1760 (2006) 1304–1313

troubleshooting:

always consider other constructs!

- **not purified, precipitating**

- chaperone co-expression
  - (3/39 structures –mar 07)
- additional purification steps
- His tag removal

- stabilisation by buffer exchange

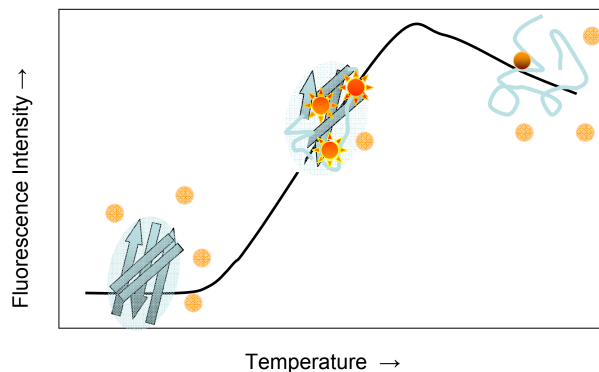
- **no or non low diffracting crystals**
- decrease dynamics, reduce surface entropy
- ligand induced stabilisation

- remove dynamic regions

- reduction of surface dynamics

our screening method: thermofluor

thermal shift assays



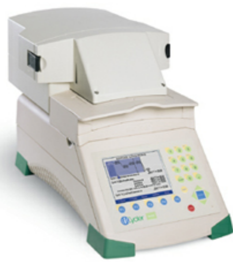
: SyproOrange fluoresce in low dielectric media (=non-polar environments such as the interior of an unfolding protein or molten globule)
λ_{ex} =490 λ_{em} =575nm

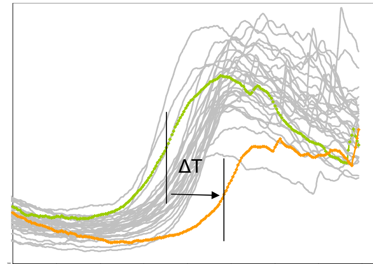
other environmental sensitive dyes:
1,8-ANS, 2,6-ANS, 2,6-TNS, Dapoxyl family

Pantoliano et al, Journal of Biomolecular Screening,2001;429-440

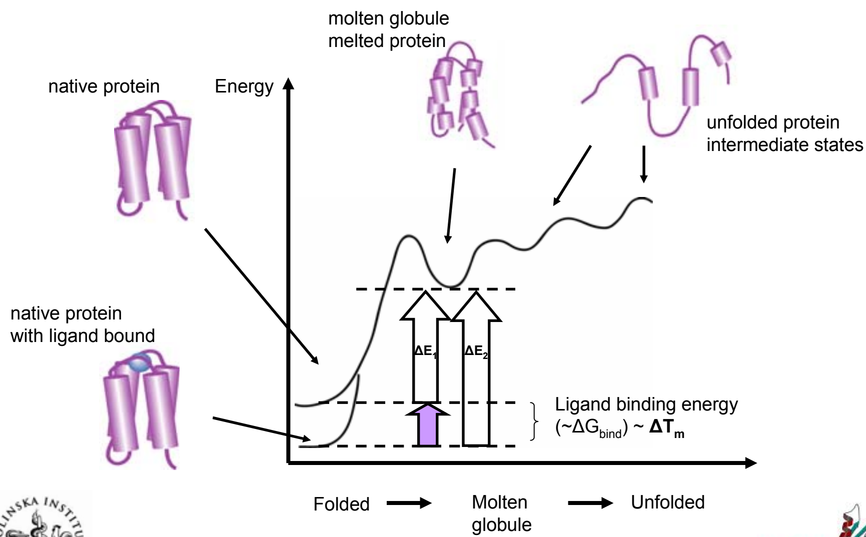
thermal shift assays

- Performed in a RT-PCR instrument:
  - 96 samples in ~1.5 hours, different buffers, ligands etc
  - ~5 µg of protein/well, ~500µg /96 well plate
  - measure fluorescence as a function of temperature in each well
  - fit each curve using a sigmoidal function
  - extract Tm, transition temperature
- ΔTm -comparison between different conditions





energetics of ligand induced conformational stabilisation

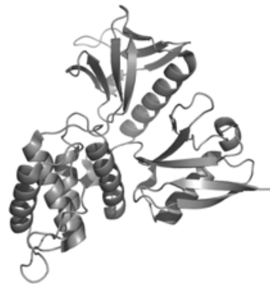


(Pantoliano et al, Journal of Biomolecular Screening,2001;429-440)

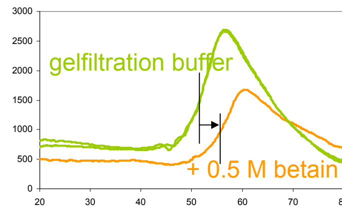
buffer screen

the FERM domain of EPB41L3 refused concentration

48 conditions: buffers, pH, salt, osmolytes, detergents



EPB41L3 (DAL-1)
2he7.pdb

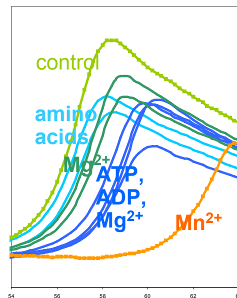


addition of 0.5 M betain enabled concentration and crystallisation

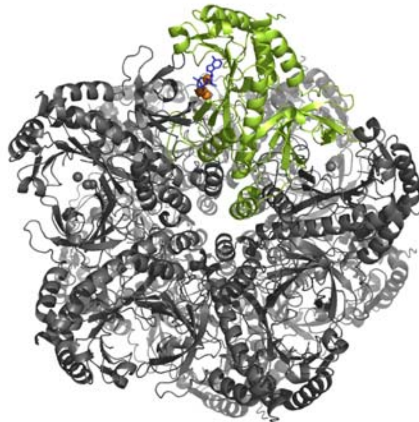
knowledge based ligand screen

an amino acid metabolic enzyme refused concentration, a literature search suggested involvement of nucleotides and ions

~20 combinations of amino acids, ions, ATP, ADP

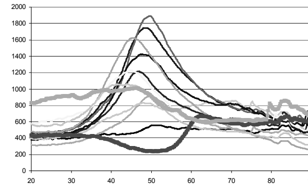


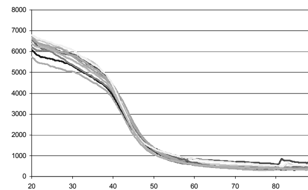
addition of ATP and Mn^{2+} enabled concentration

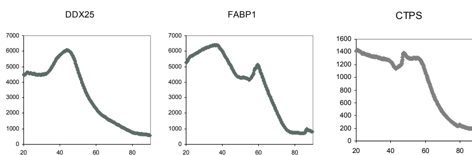


bad examples:

- non-productive experiments due to:
  - interpretation not always clear
  - aggregated states where the probe intercalates
  - hydrophobic pocket where the probe binds
  - ugly curves by well behaving proteins:







remove dynamic regions

His tag removal

•facilitate concentration and crystallisation

TEV protease

MHHHHHHSSGVDLGTENLYFQS M....

•Nov -06

~1/3 of mounted crystals have had the tag removed

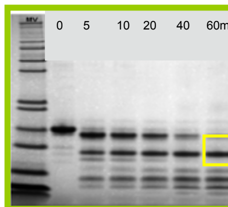
•March-07

deposited structures	39
tag removed	8
His tag in crystal cont. etc	3

limited proteolysis

•analytical tool

•preparative tool
- •identify domain borders,
- •re-engineer construct



construct identified via MS, cloned, soluble protein in small scale expression screening

reduction of surface dynamics

reductive methylation

•di-methylation of all accessible lysine side chains and the N-terminal amino group

•first attempt:

•Nov -06:

totally 9 proteins, no additional crystals, trying different protocols to minimise precipitation problems

•poster from JCSG at ICSG:

•111 proteins tested, 5 new structures, 4.5% (but said 7% in talk)

surface mutagenisis

•reduce surface entropy by mutation of charged residues to more hydrophobic

•web tool http://nihserver.mbi.ucla.edu/SER/ propose mutation candidates that are most likely to enhance crystallisability

•March -07

waiting for first set mutants

biophysics at SGC Stockholm